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No.9

CHRONIC TOXICITY OF SOME NEW INSECTICIDES TO BOB-WHITE QUAIL

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Introduction

Early pharmacological research on DDT indicated its high stability and pointed to the possibilities of the compound being accumulated and stored in body tissues. Subsequent studies have shown that other new insecticides of the chlorinated hydrocarbon group possess some of the same characteristics (Lehman, 1948). These qualities of resistance to metabolic activity and capabilities for storage in tissues increase hazards to vertebrate life beyond those that might be evident as acute poisoning effects.

Numerous field and laboratory studies by the Fish and Wildlife Service and others have shown in a general way the immediate hazards to wildlife that result from some insecticide applications. These studies have shown that control work involves dosages of DDT that are not sufficient for rapid acute poisoning of birds and mammals. However, these dosages, because of various qualities of the toxicants, and certain features of control activity, may impose hazards of chronic poisoning.

Coburn and Treichler (1946) conducted studies with DDT to determine its chronic poisoning levels for quail. The comparatively small amount of 0.025 percent of the compound administered in feed over a period of two months proved critical for 50 percent of the test animals. Over this same period some mortality resulted with the minimum amount tested (0.005 percent). At 0.40 percent, the highest concentration used in feeding tests, an acute response was found with a 100 percent kill of 10 test birds in 24 hours.

In 1947, other evaluations of new insecticides were made to determine dosage levels that result in chronic poisoning of quail. In addition to DDT, TDE or DDD (dichlorodiphenyldichloroethane), benzene hexachloride and toxaphene (chlorinated camphene) were tested. Medication was in feed at the level of 0.025 percent of each insecticide, and twenty 12-week-old quail, equally divided as to sexes, comprised a test group for each toxicant. In preliminary tests extending over 44 days, deaths attributed to insecticide poisoning were as follows: DDT, 3; TDE, 2; benzene hexachloride, 1; and toxaphene, 1. Weight records for birds in these tests failed to show any marked differences, although DDT-fed birds not only suffered the greatest mortality but also showed the least gain in weight. Additional evaluations of new insecticides in 1948-49 provide the basis for this report.

It was previously found that 0.025 percent of toxicant was not sufficiently high to provide for comparisons among compounds of lower toxicity than DDT. Also, medication at this level required dosing over rather extended periods before effects were apparent. In order to remedy these objections to the earlier routine, the concentration of toxicant in feed was increased to higher levels of 0.05 and 0.1 percent.

Methods

Tests were made with birds confined in floorless brooder coops having inside dimensions of 4 feet-9 inches by 9 feet-10 inches. Three hundred 12-week-old quail used in the studies were confined separately in groups of 20 birds (10 male and 10 female). The following seven insecticides were evaluated in the tests: DDT (dichlorodiphenyltrichloroethane), CHLORDANE (1, 2, 4, 5, 6, 7, 8, 8 - octochloro - 4, 7 - methano - 3a, 4, 7, 7a - tetrahydroindan), TDE or DDD (dichlorodiphenyldichloroethane), METHOXYCHLOR (dimethoxydiphenyltrichloroethane), TOXAPHENE (chlorinated camphene), and PARATHION (0, 0 - diethyl 0 - p - nitrophenylthiophosphate). The first five materials mentioned were used in the form of 50 percent wettable powder and tested in concentrations of 0.05 and 0.1 percent. Food mixtures of 0.05 and 0.1 percent of toxaphene were made from a 25 percent wettable powder, and those including 0.0125 and 0.025 percent of parathion were made from the pure chemical which, after being diluted with acetone, was mixed with talc. In incorporating weighed amounts of the materials in feed, the toxicant in dust form was first thoroughly mixed with a small amount (100-200 grams) of standard feed ration. This mixture was then further diluted to the desired percentage concentration by tumbling with sufficient additional feed to provide kilogram amounts.

Before beginning tests, the birds were examined for general condition and segregated into experimental lots during the dates October 18-20. From this period until October 30 normal rations of food and water were provided. Following this 10-day period of adjustment to new surroundings, the birds were weighed and experimental feeding was begun on October 31. Studies with parathion and benzene hexachloride were conducted at a later date (experimental feeding started January 26) but conditions of the experiment were essentially identical to those described above.

Results

Mortality Rates

Table 1 summarizes the mortality at the end of 8-day intervals and for a test period of over a month. It will be noted that chlordane, toxaphene, and DDT all produced a complete kill of quail at the 0.1 percent level of medication. At the end of 32 days, a 79 percent mortality was noted for birds on the diet including 0.1 percent benzene hexachloride and at 37 days the total kill was 95 percent. No losses were experienced among birds on diets including TDE, or the methoxy analog of DDT (methoxychlor). At the lower dosage of 0.05 percent, the mortality rate at 32 days was as follows: chlordane - 100 percent, toxaphene - 90 percent, DDT - 50 percent, and benzene hexachloride - no losses. Tests with benzene hexachloride extended for several days longer than for other compounds, and two losses (10 percent) were recorded on the 37th day. Animals on the TDE diet suffered no losses at the 0.05 percent concentration but one bird on the methoxychlor diet succumbed early in the test period. Since no losses with this insecticide occurred at twice this concentration, and over a longer period of time, it must be concluded that this individual was either abnormally susceptible or suffered from other deficiencies that contributed to its demise.

Tests with laboratory animals have shown that parathion is appreciably more toxic than the other compounds mentioned here (Lehman, 1949). Correspondingly, feeding tests with this material involved concentrations of one-fourth that employed in the case of the other toxicants. At levels of 0.025 and 0.0125 the kill was 89 percent and 50 percent respectively in 37 days.

Numerous studies have demonstrated that the absorption and resultant toxic action of DDT to vertebrates is enhanced by administration in oil. In acute toxic studies on quail, Coburn and Triechler (1946) found that oil solutions of DDT were 4 to 5 times as toxic as equivalent amounts of the chemical administered in crystalline form. The primarily insectivorous diet of some birds would appear to provide a source of oil which might allow for increased absorption and assimilation of DDT obtained in the course of feeding. In an effort to approximate such a condition, a diet containing 0.05 percent DDT was compared for effect with the same mixture including 0.5 percent of olive oil in addition to the insecticide. Groups of 20 birds held on each diet over a period of 37 days experienced essentially the same mortality and no difference in effect was noted. Amounts of food consumed and weight changes were comparable in the two groups.

Speed of Action

Further evidence of the relative toxicity to quail of these several insecticides may be seen in Table 2 which shows the speed with which lethal doses of the compounds were accumulated. The initial kill occurred first among DDT-fed birds, there having been two deaths on the 3rd day of feeding. A complete kill of all 20 birds required nine days on the higher level of medication with this material. Among chlordane-fed animals, initial mortalities occurred at both levels on the 4th day and the kill was complete at both levels by the 8th day. The 0.1 percent of toxaphene formulation caused the first mortality on the 8th day and a complete kill by the 13th day. At the 0.05 percent concentration, neither DDT nor toxaphene produced complete mortalities within the 32-day test period.

Studies with laboratory animals indicate that parathion is not stored in the body (Lehman, 1949). While the toxicant is not accumulative in tissues, there is no doubt but that the effects of sub-acute dosing are accumulative as is revealed by the progressive die-off of the test animals.

Weight Changes and Food Consumption

Records of weight changes of birds and their food consumption (Tables 3 and 4) suggested that some of the insecticides under test may have had a repellent effect. In the case of chlordane- and toxaphene-fed birds, the weight losses at the 0.1 percent feeding level were nearly 50 percent. That this was due to a large extent to reduced feeding is indicated by comparisons of food consumption of test and check birds. Individuals on the chlordane diet on the average consumed only about one-seventh as much feed as did the checks. Toxaphene-fed birds at the 0.1 percent feeding level ate only about one-fifth as much as the control group. Weight losses were marked among birds on the parathion diet and to a lesser extent among the animals receiving benzene hexachloride. The extent of these losses in weight (Table 3) were

reflected in records on food consumption (Table 4). Diets including DDT were accepted reasonably well and while food intake was reduced at the higher level of treatment, birds on the 0.05 percent diet ate about the same quantity of feed as did the check group. Rhothane (TDE) and methoxychlor appeared to be readily accepted by quail and diets including these compounds were utilized in about the same degree as the normal diets fed to the control birds. It should be pointed out that one of the most prominent poisoning symptoms among birds fed chlordane and toxaphene was early appearing apathy and inactivity. Consequently, it was difficult under conditions of these tests to distinguish between a repellent property of these compounds and reduced feeding that may have been due to lowered activity of the test animals.

Repellency Tests

In view of repellent properties of some insecticides as denoted by these studies on toxicity, an additional test was designed to determine more exactly the degree to which birds could detect such adulterants. Under conditions already described, individual lots of birds (10 males and 10 females) were provided a normal feed ration, and an alternate choice of the same diet which included 0.05 percent of one of the insecticides. Using separate test groups the following materials were studied for evidences of possible repellency: chlordane, toxaphene, parathion, benzene hexachloride and DDT.

During a 22-day period of study, two separate supplies of food, one treated and the other untreated, were maintained at opposite ends of the shelter. To preclude any learning mechanism from influencing results, pans of treated and untreated food were reversed frequently and at least once a day. Results of this test are summarized in Table 5. It will be noted that over a 22-day period the total (treated and untreated) food consumption by birds offered a choice of plain or insecticide-treated food was 15.2 to 16.5 grams per bird per day. A check group, subsisting only on plain food, consumed an average of 17.4 grams per bird daily. While the total food intake was comparable in the check and experimental groups, the relative amount of treated and plain foods used by the experimental birds varied widely. Individuals offered the DDT-treated diet seemed to show no discrimination between this and the unadulterated food. The total food intake included 49 percent of the treated lot and 51 percent of the untreated. The diet including benzene hexachloride was less readily accepted and only 31 percent of the total eaten was from this lot. A rather marked aversion was shown for diets including chlordane, toxaphene, or parathion, and of the total daily amounts eaten the percentage taken from treated diets amounted only to 9, 8 and 17 percent respectively.

Poisoning Symptoms and Autopsy Results

Although the behavior of affected birds on different insecticide diets was not diagnostic, certain differences seemed apparent. In the case of DDT-fed birds, the first evidence of toxic effect was nervous excitability. At the 0.1 percent level of feeding, this symptom appeared on the 3rd day of feeding. On the 4th day, characteristic tremors were observed for these birds, and on the 6th day tremors were observed among birds fed DDT at the 0.05 level. Among birds on the chlordane and toxaphene diets an almost reverse behavior

Chlordane and Toxaphene

was indicative of onset of toxic effect. As contrasted with the nervous, apprehensive, and excitable attitude of "DDT birds," these individuals were listless, slow to respond to stimuli, and remained huddled a good deal of the time. This characteristic inactivity was noted as early as the second day in the group on the 0.1 percent level of chlordane, and on the third day among those on the 0.05 percent diet. Toxaphene-fed quail exhibited almost the same symptoms beginning on the 3rd and 4th day of feeding. In advanced stages of poisoning, and just prior to death, birds of the latter group were observed to breathe heavily with frequent gasping, showed uncoordinated movement, underwent spasms, and in the final stages, kicked violently as they lay on their back. Evidence of alimentary irritation among chlordane- and toxaphene-fed birds was shown by very numerous white droppings in the pens. TDE and methoxychlor did not give rise to any behavior changes, and, as indicated above, was not lethal at the levels tested.

final clonic spasm

Since previous studies have shown the pathological picture for DDT poisoning, no further postmortem examinations were conducted on birds dying as a result of poisoning with this material. However, most of the individuals which expired following medication with other insecticides were examined. In these gross examinations no irregularities appeared with a consistency which could be considered diagnostic. However, in a high percentage of cases, both the chlordane- and toxaphene-fed birds showed extensive enteritis and congestion of the kidneys and heart. Extreme emaciation was characteristic of all victims held on these diets, as well as those fed sub-acute amounts of parathion.

Summary

The comparative toxicity for bobwhite quail of several new insecticides was determined by feeding tests. When incorporated in the diet in amounts equivalent to 0.1 and 0.05 percent, several common insecticides of the chlorinated hydrocarbon group showed the following decreasing order of toxicity: chlordane, toxaphene, DDT, benzene hexachloride, methoxychlor and TDE (Rhothane). At 0.1 percent concentration in the diet the three first-named compounds all produced a 100 percent kill in 16 days or less. The last two in the series (TDE and methoxychlor) produced no mortality over a 32-day test period. Parathion, an organic phosphate insecticide, was tested at levels of 0.025 and 0.0125 percent. Mortality of test birds over a 37-day interval was 89 percent at the higher dosage and 50 percent at the lower level.

Birds on chlordane and toxaphene diets suffered extreme weight losses which appeared to result from a marked aversion to diets containing these toxicants rather than from early toxic effects and inability to feed. Subsequent tests in which birds were provided a choice of treated and untreated feed showed that unadulterated food was used 10 to 11 times more abundantly than chlordane- and toxaphene-treated diets. No such selection was shown in tests with DDT as birds used food mixtures containing this compound in the same degree as they did the untreated food.

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Table 1. Mortality rate among groups of 20 quail which were fed rations including an insecticide in the amount shown.

Insecticide	Percent in diet	Percent mortality at the end of: 8 days	16 days	24 days	32 days	37 days
Chlordane	0.1 %	100 %	100	100	100	-
	0.05 %	100 %	100	100	100	-
Toxaphene	0.1	5	100	100	100	-
	0.05	0	25	65	90	-
DDT	0.1	95	100	100	100	-
	0.05	15	45	50	50	-
Benzene hexachloride	0.1 (19 birds)	-	-	42	79	95
	0.05	-	-	-	-	10
Methoxychlor	0.1	-	-	-	-	-
	0.05	5	5	5	5	-
TDE (Rothane)	0.1	-	-	-	-	-
	0.05(19 birds)	-	-	-	-	-
Parathion	0.025(" ")	5	37	84	84	89
	0.0125	-	10	35	45	50
Check (Untreated)	-	-	-	-	-	-

Table 2. Speed with which sub-acute dosages of insecticides produced mortality among quail.

Insecticide	Percent in diet	Number of days to: First mortality	25 percent mortality	50 percent mortality	75 percent mortality	100 percent mortality
Chlordane	0.1	4 (1)*	6	7	8	8
	0.05	4 (2)	5	6	7	8
Toxaphene	0.1	8 (1)	10	11	11	13
	0.05	11 (2)	15	19	25	--
DDT	0.1	3 (2)	4	4	5	9
	0.05	7 (1)	9	21	--	--
Benzene hexachloride	0.1	17 (1)	22	29	31	--
	0.05	33 (1)	--	--	--	--
Methoxychlor	0.1	--	--	--	--	--
	0.05	6 (1)	--	--	--	--
TDE (Rothane)	0.1	--	--	--	--	--
	0.05	--	--	--	--	--
Parathion	0.025	7 (1)	15	17	18	--
	0.0125	13 (1)	23	35	--	--
Check (Untreated)	--	--	--	--	--	--

* Figures in parentheses are the number of birds dying on the first day on which mortality was recorded.

Table 3. Weight changes among quail on diets which included one of the insecticides shown.

Insecticide	Percent in diet	Average weight (Grams per bird) At start of test	At death	No. alive at end of experiment
Chlordane	0.1	156.6	84.9 (20)*	(all dead)
	0.05	153.3	86.4 (20)	(all dead)
Toxaphene	0.1	167.5	88.8 (20)	(all dead)
	0.05	159.0	108.6 (18)	140 (2)*
DDT	0.1	142.9	151.2 (20)	---
	0.05	143.4	155.6 (10)	166.9 (10)
Benzene hexachloride	0.1	191.5 (19)	144.6 (18)	164.0 (1)
	0.05	195.8	145.5 (2)	172.3 (18)
Methoxychlor	0.1	162.5	---	180.4 (20)
	0.05	147.7	130.0 (1)	168.5 (19)
TDE (Rothane)	0.1	149.2	---	174.0 (20)
	0.05	146.9 (19)	---	174.6 (19)
Parathion	0.025	194.8 (19)	109.1 (17)	167.5 (2)
	0.0125	193.4	103.1 (10)	158.8 (10)
Check (Untreated)		138.8	---	176.5 (20)

* Figures in parentheses show number of individuals.

Table 4. Food consumption by quail on diets including the insecticide shown.

Insecticide	Percent in diet	Days of feeding to first death	Ave.daily food consumption (gms per bird) to date of first death	Days to death of last bird	Ave.daily food consumption to death of last bird	Daily food consumption to termination of test on 32nd day
Chlordane	0.1	4	2.3	8	2.6	—
	0.05	4	3.1	8	3.3	—
Toxaphene	0.1	8	3.7	13	3.7	—
	0.05	11	7.6	--	—	8.8
DDT	0.1	3	11.6	9	12.3	—
	0.05	7	18.0	--	--	18.0
Benzene hexachloride	0.1	17	11.0	--	--	10.2
	0.05	33	14.3	--	--	14.1
Methoxychlor	0.1	--	--	--	--	18.0
	0.05	6	18.0	--	--	19.4
TDE (Rothane)	0.1	--	--	--	--	18.4
	0.05	--	--	--	--	20.5
Parathion	0.025	7	7.5	--	--	9.1
	0.0125	13	9.5	--	--	9.3
Check (Untreated)	--	--	—	--	—	20.8

Table 5. Food consumption by quail on diets including insecticides.

Insecticide used in treated food	Daily food consumption (grams per bird) including untreated and insecticide treated lots	Treated food eaten (shown as percent of total food consumed)
0.05% chlordane	15.8	9
0.05% toxaphene	15.3	8
0.0125% parathion	15.8	17
0.05% benzene hexachloride	16.5	31
0.05% DDT	15.2[1/]	49
Normal diet only	17.4[2/]	--

1/ Test ran 22 days

2/ Test ran 14 days